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EFFECT OF ESSENTIAL OILS ON DRUG METABOLISM

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Abstract—A number of components of essential oils, such as menthol, α and β pinene, guaiacol and eucalyptol and the oil of *Pinus pumilio*, were studied to establish if they affect the metabolism of other drugs in rats.

Eucalyptol, administered subcutaneously or by aerosol was found to increase the *in vitro* (9000 *g*) liver metabolism of aminopyrine, *p*-nitro-anisol and aniline and *in vivo* the metabolism of pentobarbital.

RECENTLY several papers have been published about controllable factors that modify drug action and toxicity in experimental animals. Of the various environmental factors studied, the inductive activity of DDT,[1] chlordane[2] and related pesticides on microsomal enzyme systems, which is responsible for instance for the modified responsiveness of animals to barbiturates, was most dramatic. The aim of the present study was to establish if constituents of essential oils, widely employed in disinfectant sprays and air fresheners as well as in pharmaceutical preparations, affect the drug-metabolizing activity and consequently modify the pharmacological responses to drugs in rats.

MATERIAL AND METHODS

Female Sprague–Dawley rats weighing 150g ± 10 were used. Drugs were diluted in arachid oil and injected subcutaneously (s.c.) or undiluted nebulized by means of pressurized air in aerosol treatments.

The inhalation of aerosolized drugs was repeated for 4 days consecutively but for different lengths of time, as reported in the tables. The drugs were nebulized at the rate of 50 mg/min.

Two rats were placed in a round cage (4 dm^3) with solid sides for the period of inhalation.

Drugs used were: eucalyptol (cineole); guaiacol (*o*-methoxy-phenol); menthol (hexahydrothymol) (obtained from Faravelli, Milan); oil of *Pinus pumilio* (containing pinene, phellandrene, dipentene, sylvestrene and 5% of bornyl acetate) (Esperis, Milan); α-pinene and β-pinene (nopinene) (C.Erba, Milan). All products are pure according to the Italian Pharmacopeia (F.U. - 7th edition).

Pentobarbital effect (sleeping time) was evaluated as the time elapsed between loss and regaining of righting reflex. Pentobarbital concentration was determined by the method of Brodie *et al.*[3] with minor modifications.

Enzymatic activity was measured *in vitro* on 9000 *g* supernatant fraction of a liver homogenate of treated and control rats, according to Kato and Takanaka.[4] The following enzymatic reactions were estimated: ring-hydroxylation (aniline), *N*-demethylation (aminopyrine) and *O*-demethylation (*p*-nitroanisol). The metabolites,

p-aminophenol, 4-aminoantipyrine and *p*-nitrophenol respectively, formed in the incubation medium after 30 min, were analysed according to Gilbert and Goldberg.[5] The liver weight and the liver proteins were not affected by the treatment with eucalyptol aerosol.

RESULTS

Table 1 shows the results obtained with pentobarbital, 18 and 36 hr after the drug administration.

In the case of eucalyptol, there is a significant decrease in pentobarbital effect. The sleeping time of 18-hr-pretreated rats is approximately 50 per cent less than the control group. This effect is dose-dependent (Table 2), develops several hours after administration of eucalyptol and is long lasting (Table 3).

TABLE 1.

No. rats	Treatment	Interval between treatment and pentobarbital (hr)	Sleeping rats (%)	Sleeping time (min ± S.E.)
41	controls	—	80·5	36·8 ± 2
30	Eucalyptol	18	66·7	*19·6 ± 1
5	,,	36	100	*22·2 ± 3
6	Guaiacol	18	100	34·0 ± 7
6	,,	36	66	42·0 ± 1
5	Menthol	18	100	49·0 ± 5
6	,,	36	83	40·0 ± 2
6	Oil of *Pinus pumilio*	18	100	42·0 ± 4
6	,, ,, ,, ,,	36	100	38·0 ± 3
6	α-pinene	18	100	36·8 ± 3·3
6	,,	36	83	32·7 ± 4·9
6	β-pinene	18	83	34·3 ± 4
6	,,	36	100	31·9 ± 0·8

Drugs were given s.c. (500 mg/kg) before pentobarbital (25 mg/kg i.p.).
* $P < 0{\cdot}01$ vs. controls.

TABLE 2.

No. rats	Treatment	mg/kg s.c.	Sleeping rats (%)	Sleeping time (min ± S.E.)
41	controls	—	80	36·8 ± 2
10	Eucalyptol	125	30	31·7 ± 4
10	,,	250	40	*26·0 ± 1
30	,,	500	66·7	*19·6 ± 1

Pentobarbital was given at the dose of 25 mg/kg i.p. 18 hr after Eucalyptol.
* $P < 0{\cdot}01$ vs. controls.

Table 4 reports that the levels of pentobarbital in eucalyptol treated rats showing a decreased sleeping time, is significantly lower than in control rats, when it is measured in brain 90 min after pentobarbital administration.

The results reported in Table 5 show that the inhalation of aerosolized eucalyptol can reduce brain level and the narcotic effect of injected pentobarbital in rats. Agreeing with the data obtained by subcutaneous (s.c.) administration, the effect induced by the aerosol, also disappears 72 hr after the last inhalation.

TABLE 3.

No. rats	Treatment	Interval between treatment and pentobarbital (hr)	Sleeping rats (%)	Sleeping line (min ± S.E.)
15	controls		80	43·3 ± 3
15	Eucalyptol	7	100	36·8 ± 1
10	,,	18	66·7	31·6 ± 5†
5	,,	36	60	30·3 ± 3*
5	,,	48	60	44·0 ± 1

Pentobarbital was given at the dose of 30 mg/kg i.p. after eucalyptol (500 mg/kg s.c.)
*P <0·01 vs. controls
†P <0·05

TABLE 4.

No. rats	Treatment	Interval between treatment and pentobarbital (hr)	Sleeping time (min ± S.E.)	Pentobarbital concentration (g/brain)
20	controls	—	50 ± 3	16 ± 0·6
8	Eucalyptol	18	21 ± 6*	11·9 ± 1·3*
6	,,	36	29 ± 6*	12·0 ± 0·7*
9	Oil of *Pinus pumilio*	18	56 ± 6	17·5 ± 1·9
5	,, ,, ,, ,,	36	66 ± 6	17·7 ± 0·9

Drugs were given subcutaneously (500 mg/kg) before pentobarbital.
Brain concentration was measured 90 min after pentobarbital (30 mg/kg i.p.) injection.
*P <0·01 vs. controls.

TABLE 5.

No. rats	Treatment	Aerosol min of admin. in 4 days	Interval between treatment and pentobarbital admin. (hr)	Sleeping time (min ± S.E.)	Pentobarbital levels in brain (μg/g ± S.E.)
25	controls			62 ± 2	16·5 ± 0·7
8	Eucalyptol	*90	18	§24 ± 6	§7·2 ± 1·5
9	,,	90	72	57 ± 8	17·1 ± 1·2
5	,,	†30	18	§33 ± 3	§12·4 ± 0·7
9	controls			52 ± 5	15·3 ± 1
9	Oil of *Pinus pumilio*	*90	18	48 ± 4	14·4 ± 1
8	controls			42 ± 8	12·7 ± 0·9
5	α-pinene	‡60	24	33 ± 4	11·1 ± 1·5
5	β-pinene	‡60	24	35 ± 5	10·4 ± 0·3

Rats were submitted to aerosol daily for 4 days during periods of
* 15, 15, 30, 30 min respectively
† 5, 5, 10, 10 min respectively
‡ 15, 15, 15, 15 min respectively
§ P <0·001 in respect to the controls
Pentobarbital (30 mg/kg i.p.) was given at the reported hours after the last aerosol and was measured in brain 90 min after the injection.

Other constituents of essential oils, such as guaiacol, menthol, oil of *Pinus pumilio*, α- and β-pinene, are inactive on the pentobarbital sleeping time (Table 1).

Tables 4 and 5 show that brain pentobarbital concentrations are also unaffected when some of these compounds are given subcutaneously or by aerosol inhalation. The results reported in Table 6 indicate that when eucalyptol was administered by either subcutaneous injection or aerosol inhalation, 24 hr after the last administration, the activity of liver enzymes present in 9000 *g* supernatant fraction is significantly increased.

On the contrary oil of *Pinus pumilio*, that was inactive in the experiments with pentobarbital (Table 5), was also ineffective *in vitro* (Table 6).

TABLE 6.

Treatment	Way of administration	*p*-Nitrophenol (mμMole $\pm$ S.E.)	4-Amino-antipyrine (mμMole $\pm$ S.E.)	*p*-Aminophenol (mμMole $\pm$ S.E.)
controls	s.c.	225 $\pm$ 20	190 $\pm$ 20	68 $\pm$ 5
Eucalyptol	s.c.	*509 $\pm$ 59	*473 $\pm$ 72	*227 $\pm$ 16
controls	aerosol	242 $\pm$ 34	280 $\pm$ 40	88 $\pm$ 16
Eucalyptol	aerosol	*420 $\pm$ 34	*534 $\pm$ 46	*220 $\pm$ 16
controls	aerosol	265 $\pm$ 48	341 $\pm$ 77	83 $\pm$ 10
Oil of *pinus pumilio*	aerosol	278 $\pm$ 38	257 $\pm$ 31	70 $\pm$ 10

Eucalyptol was given subcutaneously (500 mg/kg) daily for 4 days. The aerosolized drugs were given daily for 4 days during periods of 15, 15, 30, 30 min respectively. The amounts aerosolized were 50 mg/min. Control rats received arachid oil subcutaneously or aerosolized. The figures are the average of six determinations. Determinations were performed 24 hr after the last administration.
* $P < 0{\cdot}01$ vs. controls.

The figures mean the metabolites formed (mμmole/g/hr) from *p*-Nitroanisol, aminopyrine, aniline respectively, used as substrates by 9000 *g* supernatant of rat liver.

DISCUSSION

In a number of components of essential oils, showing different structures such as terpenes, alcohols and phenols, eucalyptol was found to increase the activity of the microsomal enzyme systems. This effect has been demonstrated *in vitro* on different enzymatic reactions and *in vivo* on the metabolism and pharmacological activity of pentobarbital.

The results reported here, show also that eucalyptol induces the microsomal enzymes when it is given by aerosol route. It should be noted that this kind of administration is particularly used in practical medicine because of the high absorption of these drugs by mucous membranes of the respiratory tract. The possibility that terpenes are enzyme inducers had been suspected by Vessell.[7] He demonstrated that increased tolerance to barbiturates shown by mice or rats housed on softwood beddings, previously reported by Ferguson,[6] may be dependent on an increased microsomal enzyme activity displayed by these animals as compared to animals kept on hardwood beddings.[8] It was suggested that ingestion or inhalation of compounds contained in softwood may be responsible for the induction. More recently Wade *et al.*[9] showed that volatile hydrocarbons constituent of cedarwood, such as cedrol and cedrene, are effective inducers of microsomal enzymes via the inhalation route of administration.

Our results confirm this possibility and show that relatively low amounts of compounds such as eucalyptol, may affect the microsomal activity when inhaled by rats.

These data should be stressed in view of the large use of eucalyptol aerosol for therapeutic purpose and in view of the possibility that sprays of other compounds in the environment may contribute to change the drug metabolizing activity in animals.

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